REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden. to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 3. REPORT TYPE AND DATES 5/1/96 - 9/31/97 FINAL REPORT		COVERED	
4. TITLE AND SUBTITLE		J/1/30 - 3/31/3/	FINAL REPOR		DING NUMBERS
4. THE AND SOUTHER					
				NOO	0014-96-10599
Specificity and Enhanced Activity					
6. AUTHOR(S)	C 1492 4 & J At 4				
Wilfred Chen					3
7 DEDECORMING OPERANIZATION NAME(S) AND ADDRESS(FS)			O DEDE	ORMING ORGANIZATION	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 8.					RT NUMBER
College of Engineering					
University of California, Riverside					
Riverside, CA 92521					
12,010100, 011 72721					
9. SPONSORING/MONITORING AGE	ENCY NA	AME(S) AND ADDRESS(ES	Section of the sectio		SORING / MONITORING
				AGE	NCY REPORT NUMBER
Office of Naval Resea	arch				
800 North Quincy Stre	eet				
Arlington, VA 22217-	-5660				
		1			
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY S	STATEM	IFNT		12b. DIS	TRIBUTION CODE
12d. DISTRIBUTION AVAILABILITY STATEMENT					
Distribution Unlimited DESTRIBUTION STATEMENT A					
Distribution Unlimite	ed	DISTRIBUTION E	TATEMENT A		•
Distribution Unlimite	ed				
Distribution Unlimite	ed	Approved for p	ublic release		
			ublic release		
13. ABSTRACT (Maximum 200 words	ds)	Approved for p	ublic releases		
13. ABSTRACT (Maximum 200 words At the time when publ	ds)	Distribution oncern about PCBs	Unlimited contamination	in the	environment is
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is	ds) lic co also	Distribution Oncern about PCBs an increasing in	Unlimited contamination interest in the de	evelop	ment of movel
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is	ds) lic co also sses d	Distribution oncern about PCBs an increasing in that can rapidly	Unlimited s contamination the defectively	evelopm remove	nent of novel PCBs. However,
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB conge	ds) lic co also sses to	oncern about PCBs an increasing in that can rapidly are known to be	Unlimited contamination the defectively very resistant	evelopm remove to aero	ment of novel PCBs. However, bic biodegradation,
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congentarity in the interpretation of the particularly in the interpretation of the processome of the processor of the proc	is) lic co also sses t eners initia	oncern about PCBs an increasing in that can rapidly are known to be al attack by the	contamination in the de and effectively very resistant biphenyl dioxygo	evelopm remove to aero enases	ment of novel EPCBs. However, bbic biodegradation, We seek to
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the introduced broaden the substrate	ds) lic co also sses deners initia	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphe	contamination in the de and effectively very resistant biphenyl dioxygenyl dioxygenase	evelopm remove to aero enases from l	ment of novel EMPCBs. Whowever, bbic biodegradation, We seek to Pseudomonas sp.
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of de	dic co also sses d eners initia e spec egrad	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad spect	contamination in the de and effectively very resistant biphenyl dioxygenyl dioxygenyl dioxygenyl dioxygenyl my dioxygenyl	remove remove to aero enases from l	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these expenses.	ds) lic co also sses deners initia e spece	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad spectoments. In the	contamination in terest in the de and effectively very resistant biphenyl dioxygenyl dioxygenyl dioxygenase rum of PCBs, was initial round of	remove remove to aero enases from l s used DNA	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peu	ds) lic co also sses deners initia e spec egrad: xperio	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the inas LB400 and KF	contamination in terest in the de and effectively very resistant biphenyl dioxygenyl dioxygenyl dioxygenyl dioxygenyl dioxygenyl dioxygenase trum of PCBs, was initial round of 707, we were able	remove to aero enases from l s used DNA se	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl die	ds) lic co also sses to eners initia e spece egrad: xperio udomore	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the inas LB400 and KF mases with extended	contamination in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity	remove to aero enases from les used DNA se to ge towards	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4.4' congeners. One	ds) lic co also sses teners initia e spec egrad: xperio udomor oxygen	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad speciements. In the mas LB400 and KF nases with extende in particular,	contamination in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activi	remove to aero enases from l s used DNA s e to ge towards	ment of novel EPCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners.
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl dicay, 4' congeners. One Resulting analysis has	dic conservation of the special states of th	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad speciements. In the mas LB400 and KFI nases with extende in particular, yealed that rando	contamination in the de and effectively very resistant biphenyl dioxygenyl dioxygenase rum of PCBs, was initial round of 707, we were able ded specificity has equal activism shuffling of	remove to aero enases from l s used DNA s e to ge towards ity too sequence	ment of novel a PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis havenes as well as point	dic co also sses t eners initia e spec egrad: xperio udomon oxygen clone as re-	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the mas LB400 and KF1 nases with extende in particular, vealed that randotation have occur	contamination in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These nove	remove to aero enases from l s used DNA s e to ge towards ity too sequence	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners ces between the two ymes contained
At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point mutations in resulting and process and process and process are processed as a point mutations in resulting and process are processed as a point mutation and process and process are processed as a point mutation and process are processed as a point mutation and process are processed as a point mutation and processed as a point mutation and process are processed as a point mutation and p	lic co also sses t eners initia e spec egrad: xperio udomon oxygen clone as re- nt mu	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the mas LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common table tation taken the common table tab	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero from I sused DNA se to go towards ity towards en agreement	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point mutations in repriori. Additional in	lic co also sses t eners initia e spec egrad: xperio udomon oxygen clone as re- nt mu	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the mas LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common table tation taken the common table tab	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero from I sused DNA se to go towards ity towards en agreement	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a
At the time when publincreasing, there is biodegradation processome of the PCB congeparticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point mutations in resulting and process and process and process are processed as a point mutations in resulting and process are processed as a point mutation and process and process are processed as a point mutation and process are processed as a point mutation and process are processed as a point mutation and processed as a point mutation and process are processed as a point mutation and p	lic co also sses t eners initia e spec egrad: xperio udomon oxygen clone as re- nt mu	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad speciments. In the mas LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende in particular, vealed that randot tation have occurs common to LB400 and KFI nases with extende tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common to LB400 and tation have occurs to the common table tation taken the common table tab	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero from I sused DNA se to go towards ity towards en agreement	ment of novel PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point mutations in repriori. Additional indiscussed. 14. SUBJECT TERMS	dic conservations also seen ers initiate special experion oxygen as remains as remains egions resul	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad speciements. In the mas LB400 and KF mases with extende in particular, vealed that randotation have occurs common to LB400 ts from subsequents.	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero from I sused DNA se to go towards ity towards en agreement	ment of novel a PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a ling will be 15. NUMBER OF PAGES 4
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the ibroaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Perchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point mutations in repriori. Additional in discussed.	dic conservations also seen ers initiate special experion oxygen as remains as remains egions resul	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad speciements. In the mas LB400 and KF mases with extende in particular, vealed that randotation have occurs common to LB400 ts from subsequents.	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero from I sused DNA se to go towards ity towards en agreement	ment of novel a PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a ling will be
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the interest broaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point point mutations in repriori. Additional indiscussed. 14. SUBJECT TERMS DNA Shuffling, PCB, as	lic co also sses deners initia e specegrad: xperio udomon oxygen clone as re- nt mu- egion: resul-	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of biphering a broad spectoments. In the mas LB400 and KF nases with extende in particular, vealed that randot tation have occurs common to LB400 ts from subsequents.	contamination in terest in the de and effectively very resistant biphenyl dioxygenase rum of PCBs, was initial round of 707, we were able ded specificity has equal activism shuffling of cred. These novel and KF707 and at rounds of DNA	remove to aero enases from I s used DNA s e to ge towards ity too sequence el enzy cannot shuff	ment of novel a PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a ling will be 15. NUMBER OF PAGES 4 16. PRICE CODE
13. ABSTRACT (Maximum 200 words At the time when publincreasing, there is biodegradation processome of the PCB congeraticularly in the interest broaden the substrate LB 400, capable of detemplate for these exthe bphA genes of Peuchimeric biphenyl did 4,4' congeners. One Resulting analysis has genes as well as point point mutations in repriori. Additional indiscussed. 14. SUBJECT TERMS DNA Shuffling, PCB, as	lic co also sses t eners initia e spec egrad: xperio udomon oxygen clond as re- nt mu egion resul	oncern about PCBs an increasing in that can rapidly are known to be al attack by the cificity of bipheing a broad speciements. In the mas LB400 and KF mases with extende in particular, vealed that randotation have occurs common to LB400 ts from subsequents.	contamination in terest in the de and effectively very resistant biphenyl dioxygenase trum of PCBs, was initial round of 707, we were able ded specificity has equal activity me shuffling of tred. These novel and KF707 and	remove to aero enases from I s used DNA s e to ge towards ity too sequence el enzy cannot shuff	ment of novel a PCBs. However, bic biodegradation, We seek to Pseudomonas sp. as the starting shuffling between enerate various s both 2,2',5 and wards both congeners. ces between the two ymes contained be predicted a ling will be 15. NUMBER OF PAGES 4

FINAL REPORT

GRANT#: N00014-96-10599

PRINCIPLE INVESTIGATOR: Wilfred Chen

INSTITUTION: University of California, Riverside

EMAIL: Wilfred_Chen@gmail.ucr.edu

GRANT TITLE: Tuning Biphenyl Dioxygenase for Extended

Substrate Specificity and Enhanced Activity.

AWARD PERIOD: 1 May 1996 - 31 September 1997

OBJECTIVE: To generate a broad set of biphenyl dioxygenases with broadened substrate specificity and enhanced catalytic rate. We seek to apply sequential cycles of random mutagenesis with screening to identify possible structurally altered biphenyl dioxygenases with extended substrate specificity. In parallel, several chimeric dioxygenases will be created in order to explore and select for novel catalytic activity.

APPROACH: Highly chlorinated PCB congeners are known to be resistant to aerobic biodegradation, particularly the initial attack by the biphenyl dioxygenase. In addition, even minor differences in the DNA sequence affect the substrate range of the enzyme. In order to explore the sequence flexibility and to screen for novel dioxygenase with extended substrate specificity, random mutagenesis of the bphA gene coding for the large subunit of biphenyl dioxygenase from Pseudomonas LB400 will be carried out by error prone PCR. The mutant bphA fragments will be cloned into a low copy number vector and transformation into E. coli already carrying the bphEFGBC genes. Selection of clones with extended specificity towards different PCB congeners will be carried by spraying the plates with an etheral solution containing 5% of a particular congener to be screened, and incubated until formation of the yellow meta cleavage products conferred by the BphAEFGBC enzymes. This approach enables us to screen a large number of clones for extended specificity towards a large class of congeners. Promising clones from the first round of mutagenesis will be combined and further mutagenized by DNA shuffling. This approach enables us to carrying out recombination of positive clones and is much more flexible in searching the sequence space. In parallel, chimeric biphenyl dioxygenases will be generated by combining the bhpA gene from Pseudomonas LB400 with the that from Pseudomonas KF707 and KF715.

<u>ACCOMPLISHMENTS</u>: We developed a plate assay for selecting biphenyl dioxygenases with different congener specificities.

19980122 139

DTIC QUALITY INSPECTED 5

This assay was based on monitoring the formation of yellow meta cleavage products from biphenyl or PCBs. Initially, E. coli transformed with pCEC11 (plasmid pUC18 with bphEFGBC on an EcoR1-Sac1 fragment) and plasmid pCA31(plasmid pK194 harboring the chromosomal Nsi1-EcoR1 fragment of bphA gene) were sprayed with biphenyl and was shown to form yellow color on plate. Subsequently, yellow color formation was also detected towards 2,3-dichloro-biphenyl and to a lesser extend towards 2,2',5-trichloro-biphenyl, thus verifying the suitability of the screening assay.

Extensive work was invested to improve the cloning efficiency of the bphA gene derived from PCR. Primers have been designed flanking the multicloning site of the plasmid pCA31. Error prone PCR was carried using pCA31 as the template DNA. Mutation rates ranging from 0.4% to 2% were selected depending on the ratio between dATP/dGTP and the presence of manganese. The PCR fragments were purified, restricted with HindIII and EcoR1 and ligated into pK194 previously cut with the same enzymes, and transformed into E. coli previously transformed with pCEC11 to establish a mutant bphA gene library. Transformants were plated on LB-agar containing ampicillin and kanamycin as selection markers and incubated overnight at 37°C. About 75 % of these transformants turned yellow after treatment with biphenyl due to the formation of the yellow meta cleavage product. Approximately 1,000 transformants with the bphA insert was obtained per week.

We have also shown that the bphA fragment from Pseudomonas KF707 can be used to complement the bphEFGBC genes from LB400. The resulting E. coli carrying these genes turned yellow when treated with biphenyl. Moreover, yellow meta products were also detected with 4,4'-dichloro-biphenyl, a congener not degraded by LB400. The initial screening of mutant bphA fragment from LB400 will be carried out with 4,4'-dichloro-biphenyl.

In the intitial round of error-prone PCR, approximately 10,000 clones were screened and only one clone showed improved activity towards 4,4' congener. In contrast, with DNA shuffling between the bphA genes of Peudomonas LB400, KF707 and KF715, we were able to generate various chimeric biphenyl dixoygenases with extended specificity towards both 2,2',5 and 4,4' congeners (Fig. 1). Most remarkedly is clone DS13.9K which metabolized the two PCBs equally well based on the photometric measurement. To understand the molecular basis for the extended substrate range, the bphA DNA for clones # DS2.5K, DS8.5K, DS9.5K, DS10.2K, and DS13.9K was sequenced. For the first four clones only a region critical for substrate specificity was sequenced, for clone DS13.9K the entire coding region of bphA was sequenced. Though elevated activity was observed for DS2.5K, DS9.5K, DS10.2K toward 2,2',5-trichlorobiphenyl, DNA sequence comparison showed that region III and region IV were similar

with the sequence of bphA from Pseudomonas pseudoalcaligenes Random shuffling of sequences as well as point KF707. mutations have occurred. Some of the point mutations occurred in regions common to LB400, KF707, and KF715, and cannot be predicted a priori from sequence information alone. These data suggest that amino acid residues outside of region III and IV are also involved in substrate recognition. Due to high homology between the DNA sequences of the two parental genes the extend of DNA shuffling for the entire bphA gene from clone DS13.9K is difficult to estimate. However, seven point mutations were observed of which three led to amino acid substitutions. Most remarkably is the shuffling of region III which is homologous to the KF707 DNA, whereas region IV is homologous to LB400 DNA. Thus this mutant resembles the mutant BDE335-5 which was produced by site directed mutagenesis and also showed activity towards both 4,4'-dichlorobiphenyl and 2,2',5 trichlorobiphenyl. We are currently testing the mutants we have obtained so far for their ability to degrade higher chlorinated PCBs using gaschromatography. Furthermore, the mutated bphA genes can be used as parental sequences in another round of DNA shuffling to further relax the substrate spectrum of the various mutant biphenyl dioxygenases.

CONCLUSIONS: During the grant period, we have successfully demonstrated the usefulness of directed evolution towards improving aerobic PCB degradation. With the ease of the screening procedure developed, we anticipated that novel and flexible biphenyl dioxygenases with relaxed congener specificity can be created in subsequent cycles of mutagenesis.

SIGNIFICANCE: At the time when the public concern about PCBs contamination in the environment is increasing, there is also an increasing interest in developing novel biodegradadtion processes that can rapidly and effectively remove PCBs. Although many aerobic microorganisms are capable of degrading PCBs, many PCBs are resistant to the initial attack by the biphenyl dioxygenase. We have developed a directed evolution approach to fine tune the biphenyl dioxygenase not only to extend substrate specific but also to understand the structure-activity relationship of this enzyme. future enable more rational information ${\tt will}$ in the approaches to design the biphenyl dioxygenase.

AWARD INFORMATION: Dr. Wilfred Chen received the 1997 NSF Career Award.

PUBLICATIONS AND ABSTRACTS:

1. Fredi Bruhlmann and Wilfred Chen. 1997. Tuning Biphenyl Dioxygenase For Enhanced PCB Degradation. Abstr. Amer. Instit. of Chem. Engineers 1997 National Meeting.

2. Fredi Bruhlmann and Wilfred Chen, Tuning Biphenyl Dioxygenase for Enhanced PCB Degradation. Manuscript in preparation.

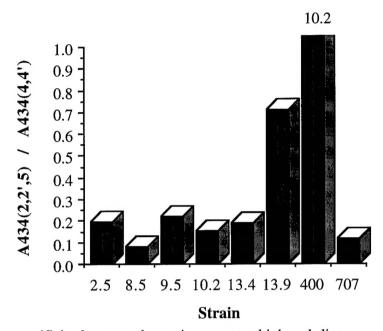


Fig. 1 Substrate specificity between the various mutant biphenyl dioxygenases and wild type biphenyl dioxygenases changed as seen by different activity ratios toward 2,2',5-trichlorobiphenyl and 4,4'-dichlorobiphenyl. Formation of the yellow meta cleavage product was measured spectrophotometrically at 434 using the cell-free culture supernatant of an overnight culture incubated with 0.5 mM of each PCB congener.